PHYTOHORMONES: STRUCTURE AND PHYSIOLOGICAL ACTIVITY. I

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In plants, growth in length by cell elongation is conditioned by certain substances elaborated in the plant and effective in minute amounts, thus having the nature of hormones. A number of simple tests have been worked out which allow of a qualitative and quantitative determination of the growth-promoting properties of various substances. With the aid of these biological tests the native growth substances or auxins have been isolated and a variety of other substances have been shown to possess growth activity. At first sight the large number of substances which are active do not appear to fall within any general classification. Upon further investigation, however, it becomes clear that there are certain common structural characteristics, and an analysis of these provides a first step in attacking the fundamental problem of the relations between chemical structure and physiological activity. This relation has in recent years been attacked in a number of other fields, usually involving physiological processes in animals. On the other hand, it would appear that plants offer an exceptionally favorable field for study in that their structures—and possibly their physiological processes—are somewhat less complicated. Thus, it has been found possible to differentiate between some of the steps in the chain of reactions which lead to cell elongation, so that now it becomes possible to determine the exact structure required in a substance for the regulation of each of these steps.

In consideration of these points experiments have already been carried out by several investigators (1–3). Our own program of research has been under way for some time with a twofold object;

on the one hand, the plant is used as a test object for bioassay to study the relation between chemical structure and physiological activity; on the other, the knowledge thus obtained is used in gaining a better understanding of the physiology of growth in plants. Thus a knowledge of the essential chemical structure of the growth substances should contribute to a knowledge of the substrates with which they react.

As was stated above, the growth of shoots of plants is specifically conditioned by certain substances, the auxins. These auxins are defined physiologically as those substances which give curvature (*i.e.*, growth promotion by cell elongation) in the standard *Avena* test (4). This test is carried out by applying the substance, dissolved in agar, to one side of a decapitated coleoptile of *Avena sativa*. The substance enters at the cut surface and moves longitudinally down that side of the coleoptile to which the agar block is applied. The growth of this side is then promoted, giving rise to a curvature, which, within limits, is proportional to the concentration of the active substance. Standard conditions have been defined and must be adhered to.

Recently another test has been described which also depends upon the promotion of growth on one side of the object. It consists of immersing 4 cm. sections of the etiolated stem of *Pisum* seedlings, longitudinally slit down the center, in the test solution. In active solutions the two halves of the stem curve towards one another, and in this case the curvatures are proportional, within limits, to the logarithm of the concentration of the active substance. All physiological details and literature of both these tests have recently been summarized elsewhere (4). In this "pea test," by contrast with the *Avena* test, the substance does not have to move longitudinally in the tissue but enters everywhere from the solution. Both tests, however, depend primarily upon asymmetrical promotion of growth, in the former by asymmetric application, in the latter by asymmetric response.

Any substance active in the *Avena* test is also active in the pea test; however, a substance active in the pea test may lack certain properties, such as sufficient transportability, and hence be incapable of causing normal curvature in the *Avena* test. Thus the two tests differ in specificity, and a substance possessing growth-promoting activity ("primary activity") is not neces-

sarily active on *Avena* (1). For the sake of simplicity, however, we have considered in this paper only the presence or absence of primary growth-promoting activity; *i.e.*, activity in the pea test. The complications of secondary properties, which allow of the causation of curvature on *Avena*, and probably also affect the quantitative relations in the pea test, will be dealt with in a later paper. In this, the first of a projected series of reports, an attempt is therefore made to present the minimum structural requirements for growth activity.

All the new substances tested for primary activity, and new tests[1] for primary activity of substances previously tested by other techniques, are listed in Table I with an identification number in roman numerals. Table I includes a reference to the method of preparation, or, in the case of substances obtained from other laboratories, an acknowledgment of their source together with an approximate estimate of the activity, expressed as per cent of that of 3-indoleacetic acid. All acids were dissolved in water with the addition of an equivalent amount of NaOH and buffered to pH 5 with diphthalate buffer.

1. The Nucleus

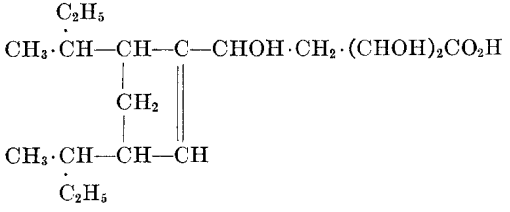
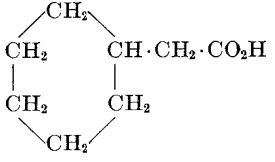
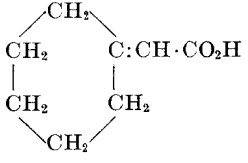
Ring—The active compounds so far known can be classified into a number of groups according to the nucleus of the molecule. The following are examples of active substances in which a ring is present: auxin *a* (II), Δ^1-cyclohexeneacetic acid (VII), α-toluic acid (phenylacetic acid) (IX), 3-indeneacetic acid (1), α-naphthaleneacetic acid (XXVII), acenaphtheneacetic acid (29), anthraceneacetic acid (XXVIII), fluoreneacetic acid (29), 1-benzofuraneacetic acid (1), 2-benzofuraneacetic acid (XXIX), 3-indoleacetic acid (heteroauxin) (XXXIII), and 2-thionaphtheneacetic acid (30). From the foregoing it is evident that homocyclic and heterocyclic 5- or 6-membered rings may be present in active substances.

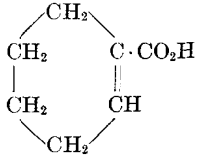
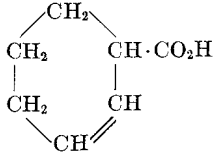
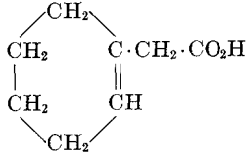
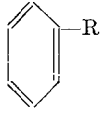
In order to determine whether the presence of a ring system is itself essential for growth activity, it is necessary to know what the other structural requirements are. From the considera-

[1] The biological data were obtained with the assistance of work Project No. 6062, Official Project No. 65-3-5380, conducted under the auspices of the Works Progress Administration.

TABLE I

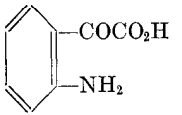
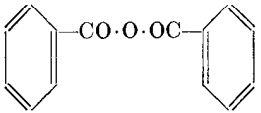
Summary of New Results, Obtained with Pea Test

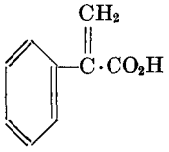
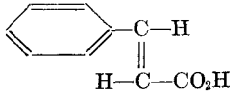
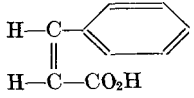
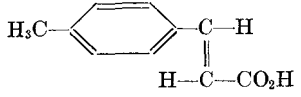
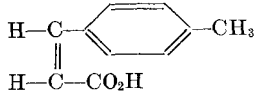
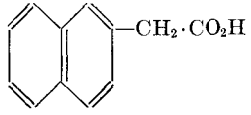
Identification No.	Compound	Source (bibliographic reference No.)	Activity expressed as per cent of that of 3-indoleacetic acid	Remarks
I	$CH_2{:}CH{\cdot}CH_2{\cdot}CO_2H$	(5)	Inactive, 0.2 mg. per ml.	
II		*	100	For *Avena* test see (6)
III		(7)	Inactive, 0.9 mg. per ml.	
IV		(7)	" 0.5 " " "	

V		(8)	Inactive, 1 mg. per ml.	
VI		(9)	" 1 " " "	
VII		(10)	5	*Cf.* (III)
VIII	 R = —CO_2H		Inactive, 1 mg. per ml.	
IX	" = —$CH_2 \cdot CO_2H$		2	Confirming (3)

* Gift of Professor Kögl of Utrecht.

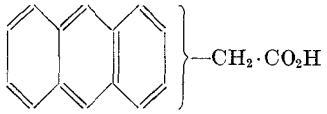
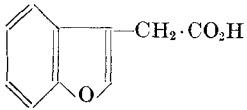
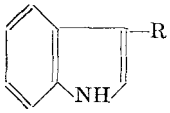
TABLE I—*Continued*

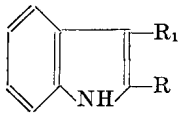
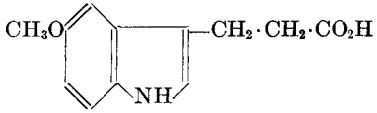
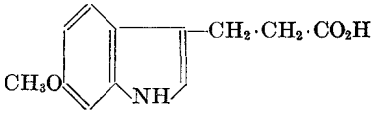
Identification No.	Compound	Source (bibliographic reference No.)	Activity expressed as per cent of that of 3-indoleacetic acid	Remarks
X	R = —$CH(CH_3)\cdot CO_2H$	(11)	6	
XI	" = —$C(CH_3)_2\cdot CO_2H$	(12)	Inactive, 0.7 mg. per ml.	
XII	" = —$CH_2\cdot CH_2\cdot CO_2H$		1	Confirming (3)
XIII	" = —$CH_2(CH_2)_2\cdot CO_2H$	(13)	Inactive, 0.8 mg. per ml.	
XIV	" = —$CH_2(CH_2)_3\cdot CO_2H$	(14)	" 0.5 " " "	
XV	" = —$CHOH\cdot CO_2H$	†	" 0.5 " " "	Also *d* and *l* form
XVI	" = —$C(CH_3)OH\cdot CO_2H$	†	" 0.5 " " "	" " " " " Reported weakly active (3)
XVII	" = —$CH(CH_2\cdot OH)\cdot CO_2H$		" 0.9 " " "	*dl* form
XVIII	" = —CH_2COCH_3		" up to saturated solution	*Cf.* (IX)
XIX	" = —CH_2CONH_2		" "	" (IX)
XX		(15)	2	Lower activity than reported (3)
XXI			Inactive up to saturated solution	Reported active on *Avena* (16)

XXII		(17)	2	
XXIII			Inactive, 0.7 mg. per ml.	
XXIV		(18)	10	Lower activity than reported (3); *cf.* (XXIII)
XXV		(19)	Inactive, 0.3 mg. per ml.	Confirming (3)
XXVI		(20)	10	*Cf.* (XXV)
XXVII		‡	100	

† Gift of Professor Alexander McKenzie, University College, Dundee.
‡ Gift of P. W. Zimmerman, Boyce Thompson Institute.

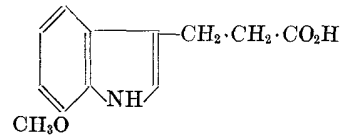
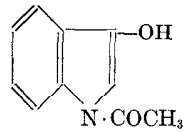
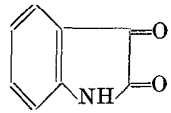
TABLE I—*Continued*

Identification No.	Compound	Source (bibliographic reference No.)	Activity expressed as per cent of that of 3-indoleacetic acid	Remarks
XXVIII		‡	*100*	
XXIX		§	*15*	
				
XXX	R = —CH_3	(21)	Inactive, 0.2 mg. per ml.	Reported active on *Avena* (22)
XXXI	" = —OH	(23)	"	*Cf.* Section 2
XXXII	" = —CO_2H	(24)	" 0.2 mg. per ml.	Confirming (3)
XXXIII	" = —$CH_2 \cdot CO_2H$	(25)	100	Standard
XXXIV	" = —$CH(CH_3) \cdot CO_2H$	(2)	100	
XXXV	" = —$CH_2 \cdot CH_2 \cdot CO_2H$	(26)	100	Confirming (4)
XXXVI	" = —$CH_2(CH_2)_2 \cdot CO_2H$	‖	100	" (4)
XXXVII	" = —$CH_2(CH_2)_3 \cdot CO_2H$	‖	50	

XXXVIII	R = —CH(CH$_2$·CO$_2$H)·CO$_2$H 	‖	Inactive, 0.2 mg. per ml.	
XXXIX	R = —CO$_2$H R$_1$ = —H	(27)	Inactive, 0.2 mg. per ml.	
XL	R = —CO$_2$H R$_1$ = —CH$_2$·CH$_2$·CO$_2$H	‖	" 0.2 " " "	
XLI	R = —CO$_2$H R$_1$ = —CH$_2$(CH$_2$)$_2$·CO$_2$H	‖	" 0.2 " " "	
XLII	R = —CO$_2$H R$_1$ = —OH	(28)	"	
XLIII		‖	" 0.2 mg. per ml.	*Cf.* (XXXV)
XLIV		‖	" 0.2 " " "	" (XXXV)

§ Gift of Professor T. Reichstein of Zürich.
‖ Gift of Dr. R. H. F. Manske, National Research Council, Ottawa.

TABLE I—*Concluded*

Identification No.	Compound	Source (bibliographic reference No.)	Activity expressed as per cent of that of 3-indoleacetic acid	Remarks
XLV		‖	Inactive, 0.2 mg. per ml.	*Cf.* (XXXV)
XLVI		(23)	" 0.1 " " "	
XLVII			" up to saturated solution	*Cf.* (XX)

tions advanced below it is clear that 3-butenoic acid (I) would possess all the characteristics known to be needed for activity with the exception of a ring. This substance was therefore prepared and found to be completely inactive. This, together with the fact that up to now not a single aliphatic acid has been shown to have true growth activity (see the list of compounds found inactive in (3)), strongly indicates that a ring structure is essential.

Double Bond—With saturation of the double bond of auxin *a* (II) and auxin *b* (6) or of the $\Delta^{2,3}$ double bond of 3-indoleacetic acid (XXXIII) (2), all activity on *Avena* disappears. In general only substances possessing an unsaturated ring system have activity. A test of this requirement is given by the series of comparable substances, cyclohexaneacetic acid (III), cyclohexylideneacetic acid (IV), and Δ^{1}-cyclohexeneacetic acid (VII), of which the last mentioned substance, which has the double bond in the ring, alone possesses activity. This strikingly parallels the inactivity of dihydroauxin *a* and pseudoauxin *a*, and the activity of auxin *a*, as described by Kogl and coworkers (31).

Substituents—In the present investigation, the effect of nuclear substituents other than the side chain bearing a carboxyl group has not been sufficiently studied to warrant any new conclusions. However, it may be pointed out that the introduction of a methyl group into the 1-, 2-, or 5-position of 3-indoleacetic acid (XXXIII) did not render the resulting compounds inactive (3), while the replacement of the methyl group by an ethyl group in the 2-position of 3-indoleacetic acid gave the inactive 2-ethyl-3-indoleacetic acid (3). Likewise the introduction of the methoxyl group into active 3-indolepropionic acid (XXXV) gave the inactive 5-, 6-, and 7-methoxy-3-indolepropionic acids (XLIII, XLIV, XLV). In the benzene series the amino group has without doubt an important effect. Thus phenylglyoxylic acid (3) is inactive but *o*-aminophenylglyoxylic acid (isatinic acid) (XX) is active. An amino-substituted α-toluic acid, namely *p*-amino-α-toluic acid, has been reported as active in the pea test (3).

2. The Side Chain

Carboxyl Group—Up to the present almost all of the active substances known are either carboxylic acids or their esters. The

exceptions noted are tryptamine (32), α-naphthaleneacetonitrile (29), and auxin *a* lactone (6). Satisfactory evidence for the conversion of tryptamine by the *Avena* coleoptile into an active acid, based essentially upon the fact that its action is delayed, has been given by Skoog (32). Unpublished data indicate that tryptophane behaves in the same way. Similar evidence for the conversion of α-naphthaleneacetonitrile has been mentioned by Zimmerman and Wilcoxon (29), though on different plant material, and the activity on *Avena* of auxin *a* lactone may safely be ascribed to its hydrolysis in the plant.

In view of the inactivity of indole, the reported activity on *Avena* of skatole (XXX) by Glover (22) seemed questionable, particularly since skatole may be prepared by the decarboxylation of 3-indoleacetic acid. Skatole was therefore prepared by the Fischer synthesis to avoid the possibility of active contaminants, and found to be completely inactive. It would thus seem that the activity of Glover's specimen of skatole must be ascribed to an impurity.

Other indole derivatives lacking a carboxyl group, namely indoxyl (XXXI), 1-acetylindoxyl (XLVI), and isatin (XLVII) are also inactive. That the inactivity of indoxyl was not due to its failure to enter was shown by the observation that after application, either in agar or in solution, the plant showed a blue precipitate of indigo within the cells over a length of about 1 mm. In this connection it may further be noted that benzyl methyl ketone (XVIII), which carries a methyl group in place of the hydroxyl group of the active α-toluic acid, does not have the slightest activity.

The activity of esters is of considerable interest. Kogl and coworkers showed that the methyl ester of auxin *a* (II) is inactive on *Avena* (6); the esters of 3-indoleacetic acid (XXXIII), on the other hand, are active on *Avena*. This activity has been ascribed by Kogl and Kostermans (2) to the ready hydrolysis of the esters by plant lipases. It is certainly significant that there is no known instance of an inactive carboxylic acid whose ester possesses growth activity (2, 3, 33).

Position of Carboxyl Group—There are no special limitations which may be placed on the length of the side chain which carries the carboxyl group. This is evidenced in the indole series by

the activity of 3-indoleacetic acid (XXXIII), α-methyl-3-indoleacetic acid (XXXIV), 3-indolepropionic acid (XXXV), 3-indolebutyric acid (XXXVI), and 3-indolevaleric acid (XXXVII). In the benzene series only α-toluic acid (IX), hydratropic acid (X), and hydrocinnamic acid (XII) are active; the higher homologues, γ-phenylbutyric (XIII) and δ-phenylvaleric acid (XIV), were inactive. However, the carboxyl group must be removed at least 1 carbon atom from the ring, because the following substances are inactive: Δ^1-cyclohexenecarboxylic acid (V), Δ^2-cyclohexenecarboxylic acid (VI), benzoic acid (VIII), 2-indolecarboxylic acid (XXXIX), 3-indolecarboxylic acid (XXXII) (25), and indoxylic acid (3-hydroxy-2-indolecarboxylic acid) (XLII). An exception to the above rule was the reported activity of dibenzoyl oxide and dibenzoyl peroxide (XXI) on *Avena* by Snow (16). The latter substance when tested in these laboratories, however, was completely inactive in the pea test and caused a growth inhibition of *Avena*, a phenomenon unrelated to the growth activity under consideration here.

Although the presence of a carboxyl group (or a group readily hydrolyzable to a carboxyl group by the plant) is necessary for growth activity, the presence of more than one appears, in certain instances, to abolish activity. Examples of inactive dicarboxylic acids are 2-carboxy-3-indolepropionic acid (XL), 3-indolesuccinic acid (XXXVIII), and 2-carboxy-3-indolebutyric acid (XLI). The dicarboxylic acids or esters reported active thus far are *m*-phenylenediacetic ethyl ester (3), and indylene-1,3-diacetic acid (34).

Double Bond—The following two compounds which have a double bond in the side chain as well as an unsaturated ring are active, *cis*-cinnamic acid (XXIV) (3) and atropic acid (XXII). The corresponding analogues with a reduced side chain, namely hydrocinnamic acid (phenylpropionic acid) (XII) (3) and hydratropic acid (X), are likewise active. Thus the degree of unsaturation of the side chain does not appear to be important, at any rate for qualitative activity.

Substituents—The available experimental evidence does not warrant any conclusions with regard to the effect of substituents in the side chain, but the experimental data thus far obtained will be reported at this time. Examples of methyl substitution

in the acetic acid side chain of the benzene series are the active hydratropic acid (X), and the inactive α,α-dimethyl-α-toluic acid (XI). In the indole series α-methyl-3-indoleacetic acid (XXXIV) is active but unfortunately attempts to prepare α,α-dimethyl-3-indoleacetic acid have thus far been unsuccessful.

In view of the fact that the active auxins *a* (II) and *b* have four and two hydroxyl groups respectively in the side chain carrying the carboxyl group, the inactivity of *d*- and *l*-mandelic acid (XV), *d*- and *l*-atrolactic acid (XVI), and *dl*-tropic acid (XVII) is quite remarkable. This fact is of especial interest in that the replacement of the hydroxyl group in each of the inactive compounds mentioned above with hydrogen results in the active α-toluic acid (IX) in the first instance and the active hydratropic acid (X) in the case of the atrolactic and tropic acids.

Space Configuration

The importance of *cis-trans* isomerism is clearly indicated by the following relationships.

Active	Inactive
cis-Cinnamic acid (XXIV) (3)	*trans*-Cinnamic acid (XXIII) (3)
cis-p-Methylcinnamic acid (XXVI)	*trans-p*-Methylcinnamic acid (XXV)
cis-o-Methoxycinnamic acid (3)	*trans-o*-Methoxycinnamic acid (3)

The *trans* modifications of *o*-, *m*-, and *p*-nitrocinnamic acid were tested and found to be inactive in the pea test.

DISCUSSION

The activity of the plant hormones in causing growth may be considered in terms of their chemical structure as it affects the physical properties of the molecule and as it affects the chemical reactivity of the molecule. The direct proportionality between auxin applied and growth induced is good *a priori* evidence that a stoichiometric relation exists and confirmation of this view has been obtained from the pea test described above. Examination of the lowest active concentrations of 3-indoleacetic acid, 3-indolepropionic acid, 3-indolebutyric acid, and α-naphthaleneacetic acid and auxin *a* has shown that mole for mole their activity is almost the same (4). This strongly indicates that these substances combine directly with some substance in the cell. These facts directed the course of this investigation toward

a correlation of physiological activity with chemical structure rather than with the physical properties of growth-promoting substances. As stated before, the problem has been simplified by not considering the quantitative relationship or degree of activity, which to some extent may perhaps be due to secondary properties (including physical properties).

If the problem is thus simplified and the question limited to one of whether a substance possesses or does not possess the ability to cause cell elongation in plants, then the minimum structural requirements for activity as indicated by the experimental evidence so far reported are (*a*) a ring system as nucleus, (*b*) a double bond in this ring, (*c*) a side chain, (*d*) a carboxyl group (or a structure readily converted to a carboxyl, such as an ester or nitrile) on this side chain at least 1 carbon atom removed from the ring, (*e*) a particular space relationship between the ring and the carboxyl group.

The question of space relationship appears to be one of the most important in view of the clear cut evidence offered by the *cis*- and *trans*-cinnamic acids (XXIV, XXIII). The most obvious difference between the *cis*- and *trans*-isomers is the distance between the carboxyl group and the nucleus and this suggests that the growth activity of the *cis*-isomers is occasioned by the close proximity of the carboxyl group to the nucleus. Thus in the α-toluic acid series of active compounds steric hindrance produced by the introduction of two methyl groups on the α-carbon atom might account for the inactivity of α,α-dimethyl-α-toluic acid (XI).

The importance of the space relationship is perhaps supported further by the inactivity of the dicarboxylic acids, 2-carboxy-3-indolepropionic acid (XL) and 2-carboxy-3-indolebutyric acid (XLI) (*cf.* Section 2). Here also steric hindrance could well prevent the requisite space relationship between the carboxyl group of the side chain and the nucleus in these compounds. Likewise, the inactivity of 2-ethyl-3-indoleacetic acid in contrast to the activity of 2-methyl-3-indoleacetic acid might be ascribed to greater steric hindrance exerted by the ethyl group in the 2-position than by a methyl group in the same position.

In studying the characteristics common to all active substances, we have considered the possibility that an active hydrogen atom

must be present in the side chain bearing the carboxyl group. This would give an obvious explanation for the inactivity of the ring-substituted carboxylic acids such as benzoic and the indolecarboxylic acids, as well as for the striking inactivity of α,α-dimethyl-α-toluic acid as contrasted with the activity of hydratropic acid. However, the undoubted activity of isatinic acid (XX) is in conflict with this hypothesis. It is possible that active hydrogen atoms are in fact present in the side chain of isatinic acid, either by hydration of the ketone group, or, more probably, by exchange of hydrogen atoms with those in the ring, involving formation of a quinonoid structure. Some such interchange appears to be indicated by the fact that the presence of the *o*-amino group is necessary for activity to be shown, for phenylglyoxylic acid is inactive. For the present, however, this explanation must be left open.

The activity of apparently wholly unrelated substances such as auxin *a* (II), *cis*-cinnamic acid (XXIV), and 3-indoleacetic acid (XXXIII) has given the impression that no structural specificity exists. From the above analysis it would seem that this view has resulted from the fact that some of the structural requirements of the molecule for growth-promoting activity are comparatively simple, but at the same time it appears that they must be strictly adhered to whether present in a large complex molecule or in a smaller and simpler one. Thus by adhering to the usual chemical classification of organic substances based, for example, on the nucleus, structural specificity in physiological reactions may be lost sight of, although in reality activity evidently depends upon a *combination* of structural characteristics. A striking example of this thesis is afforded by the apparent lack of chemical relationship in the estrogenic substances (35). A parallel may be drawn in another respect between estrogenic and plant growth substances. In both cases there are instances of highly active substances of comparatively simple structure, whereas the naturally occurring estrone and auxin *a* have much more elaborate molecules. Thus the complexity of the molecule is not necessarily connected with its primary activity. In the case of the auxins evidence will be given in later reports that the complexity of the molecule is connected with its secondary properties.

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SUMMARY

The specificity of the growth- (cell elongation) promoting properties of a number of synthetic substances have been determined by the use of simple biological tests and evidence is presented that the specificity of physiological activity does not necessarily depend upon the nucleus of a substance but upon a particular molecular configuration. The minimum structural requirements for cell elongation activity in higher plants as indicated by the experimental evidence reported are (*a*) a ring system as nucleus, (*b*) a double bond in this ring, (*c*) a side chain, (*d*) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least 1 carbon atom removed from the ring, and (*e*) a particular space relationship between the ring and the carboxyl group.

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